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SUPPLEMENTARY ONLINE DATA

# Glutathionylation of cytosolic glyceraldehyde-3-phosphate dehydrogenase from the model plant *Arabidopsis thaliana* is reversed by both glutaredoxins and thioredoxins *in vitro*

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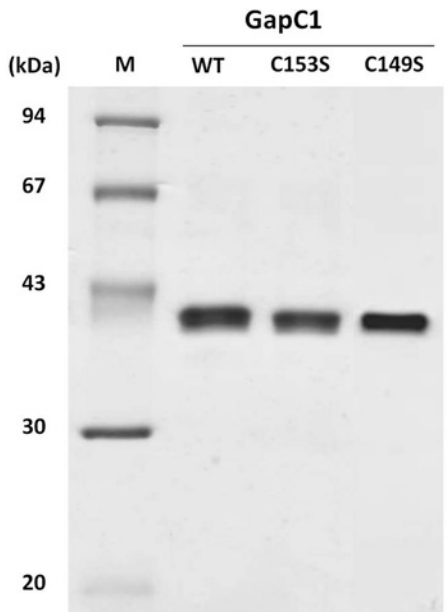


**Figure S1 SDS/PAGE of wild-type GapC1 and mutants C153S and C149S of *A. thaliana* expressed in *E. coli* and purified to homogeneity**

Sample proteins (2 $\mu$g) were separated by 12 % polyacrylamide gel and stained with Coomassie Brilliant Blue. M, marker; WT, wild-type. Molecular mass in kDa is shown.

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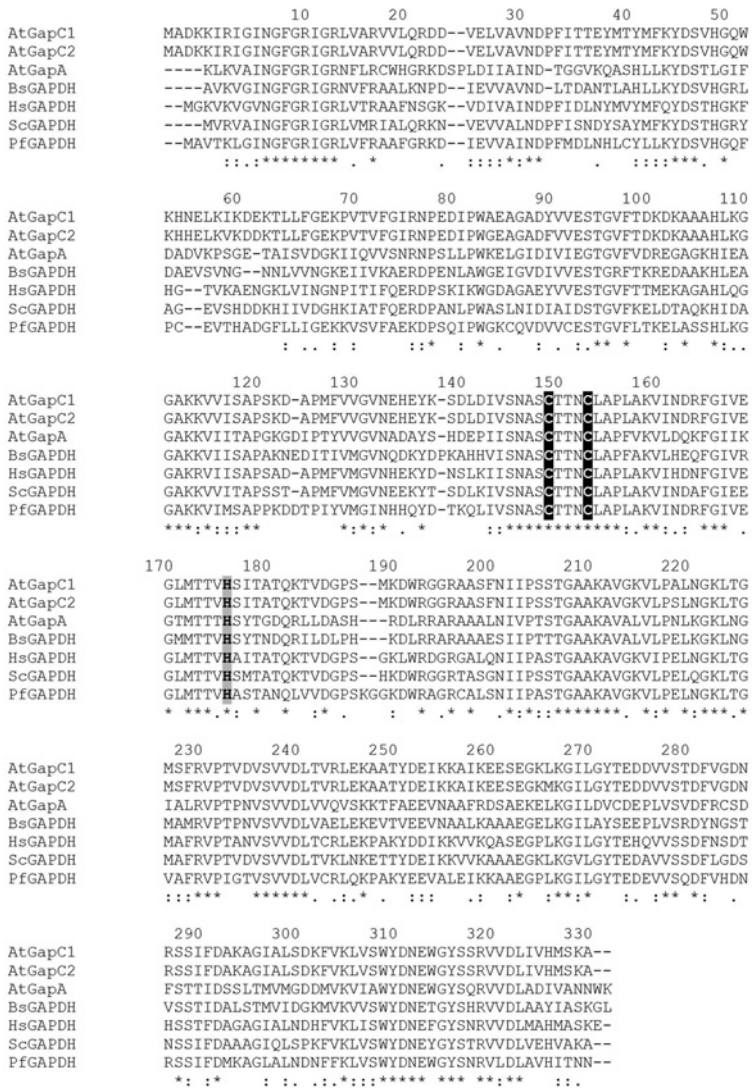


**Figure S2 Multiple alignment of GAPDH from diverse organisms**

The proteins were aligned with the ClustalW2 program and corrected manually for N-terminal extensions. Residues are numbered according to the structure of *B.stearothermophilus* GAPDH deposited in the Protein Data Bank (PDB code 2DBV; [34]). AtGapC1 (NCBI accession number AEE74039.1), AtGapC2 (NCBI accession number AEE29016.1) AtGapA, (NCBI accession number AEE77191.1), *B. staerothermophilus* BsGAPDH (PDB code 2DBV), *Homo sapiens* HsGAPDH (NCBI accession number P04406.3), *S. cerevisiae* ScGAPDH (NCBI accession number P00358.3) and *Plasmodium falciparum* PfGAPDH (NCBI accession number XP_001348772.1). $Cys^{149}$ and $Cys^{153}$ residues are on a black background, whereas $His^{176}$ is on a grey background. *, invariant residues; ., conservation between residues with weakly similar properties; :, conservation between residues with strongly similar properties.

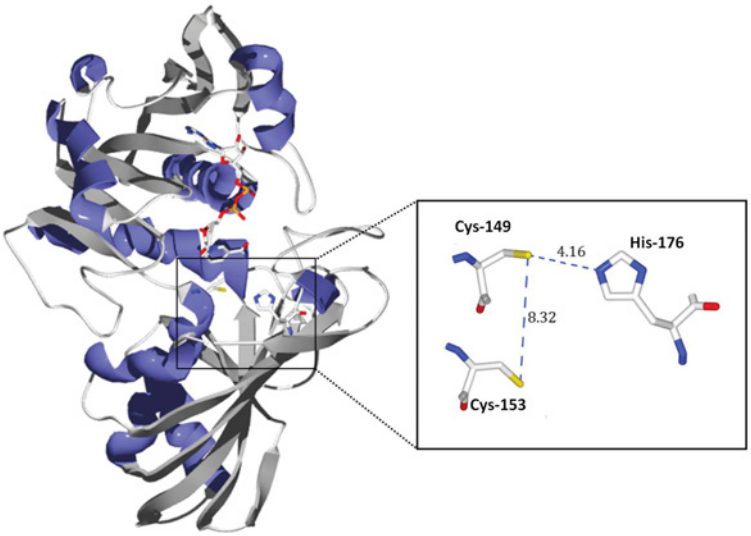


**Figure S3 Three-dimensional model of *A. thaliana* GapC1**

The model was made using Swiss-Model workspace (http://swissmodel.expasy.org/workspace/) based on the known structure of GAPDH from *Oryza sativa* (PDB code 3E5R). The structure was generated with the Swiss-PDB viewer software and rendered with POV-Ray (http://www.povray.org). The $NAD^+$ cofactor and $Cys^{149}$, $Cys^{153}$ and $His^{176}$ are shown. Inset: blue broken lines represent the distance (in Å) between $Cys^{149}$ and $Cys^{153}$ and between $Cys^{149}$ and $His^{176}$.

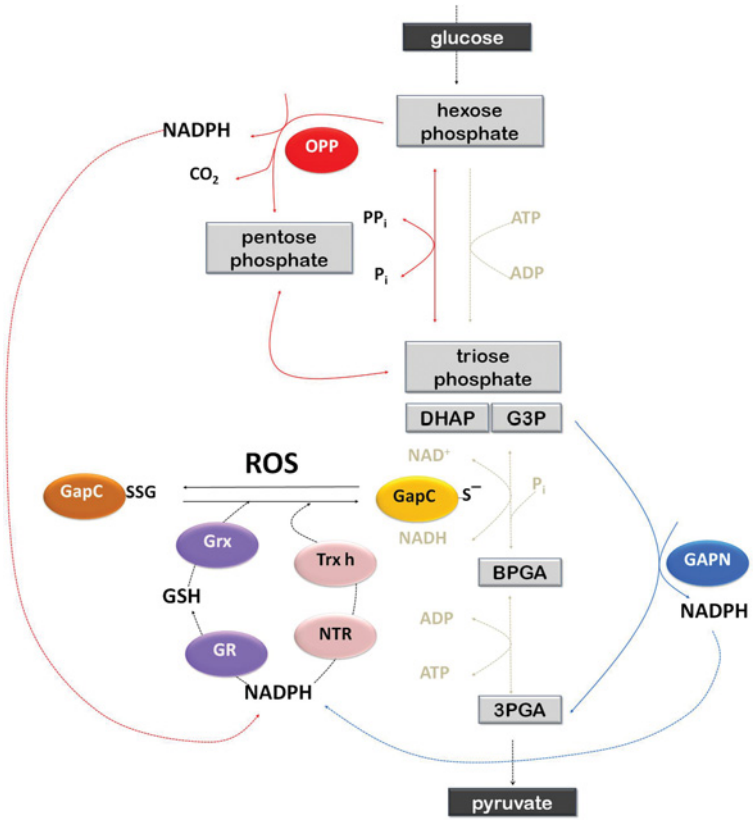


**Figure S4 Schematic representation of glycolysis showing the NADPH-producing systems in a situation of GapC glutathionylation**

Under stress conditions, GapC might undergo glutathionylation with important effects on cytosolic primary metabolism. Indeed, inhibition of GapC activity and the consequent down-regulation of the glycolysis pathway would promote entry of glucose equivalents into the OPP (oxidative pentose phosphate) pathway leading to the generation of NADPH (red arrows). Although inhibition of GapC would down-regulate the glycolytic pathway, plant cells also contain a non-phosphorylating GAPDH (GAPN) that can bypass the GapC-catalysed reaction, providing an alternative source of NADPH for the antioxidant enzymes (blue arrows). GR and NTR are major antioxidants enzymes in the cytosol of plant cells. GR, using NAPDH as an electron donor, can keep the glutathione pool reduced, providing the reductant (GSH) for the efficient deglutathionylation of GapC via cytosolic glutaredoxins. Alternatively, GapC1 may be also deglutathionylated by a GSH-independent system involving NADPH, NTR and cytosolic Trxh. Overall, redirection of primary metabolism in stressed plant cells would allow reinforcing the antioxidant systems and creating the conditions for recovery (e.g. reduction/reactivation of glutathionylated proteins such as GapC1). DHAP, dihydroxyacetone phosphate; 3PGA, 3-phosphoglycerate.

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